Copy for the Elected Office (EO/US)

PCT/IB98/00821

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

ROBERTS, T., W.
Batchellor, Kirk & Co.
102-108 Clerkenwell Road
London EC1M 5SA
ROYAUME-UNI

Date of mailing (day/month/year)
27 September 1999 (27.09.99)

Applicant's or agent's file reference
TR/RM/97058W

IMPORTANT NOTIFICATION

International application No.
PCT/IB98/00821

International filing date (day/month/year)
14 May 1998 (14.05.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

D.J. VAN DER HAVE B.V.
Dijkwelsestraat 70
NL-4420 AA Kapelle
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

ADVANTA SEEDS B.V.
Dijkwelsestraat 70
NL-4420 AA Kapelle
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 January 1999 (12.01.99)	
International application No. PCT/IB98/00821	**Applicant's or agent's file reference** TR/RM/97058W
International filing date (day/month/year) 14 May 1998 (14.05.98)	**Priority date** (day/month/year) 14 May 1997 (14.05.97)
Applicant SMEEKENS, Sjef et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 December 1998 (10.12.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer P. Regis
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

ROBERTS, T., W.
Batchellor, Kirk & Co.
102-108 Clerkenwell Road
London EC1M 5SA
ROYAUME-UNI

Date of mailing (day/month/year)
05 May 1999 (05.05.99)

Applicant's or agent's file reference
TR/RM/97058W

IMPORTANT NOTIFICATION

International application No.
PCT/IB98/00821

International filing date (day/month/year)
14 May 1998 (14.05.98)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

ROBERTS, T., W.
Batchellor, Kirk & Co.
2 Pear Tree Court
Farringdon Road
London EC1R 0DS
United Kingdom

State of Nationality

State of Residence

Telephone No.
44 171 253 1563

Facsimile No.
44 171 253 1214

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

ROBERTS, T., W.
Batchellor, Kirk & Co.
102-108 Clerkenwell Road
London EC1M 5SA
United Kingdom

State of Nationality

State of Residence

Telephone No.
44 171 253 1563

Facsimile No.
44 171 253 1214

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

P. Regis

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION (see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below)	
TR/RM/97058W		
International application No	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/IB 98/00821	14/05/1998	14/05/1997
Applicant		
D.J. VAN DER HAVE B.V. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application.

   [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority.

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/00821

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N15/11 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAUL R. ROBSON ET AL: "Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene." NATURE BIOTECHNOLOGY, (1996) 14/8 (995-998). ISSN: 0733-222X CODEN: NABIF. XP002075914 cited in the application	22,23
A	see the whole document	24

-/--

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

Special categories of cited documents

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

& document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1998	21/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax (+31-70) 340-3016	Kania, T

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	QUAEDVLIEG N ET AL: "The homeobox gene ATH1 of Arabidopsis is derepressed in the photomorphogenic mutants cop1 and det1." PLANT CELL. (1995 JAN) 7 (1) 117-29. JOURNAL CODE: BJU. ISSN: 1040-4651.. XP002075915 cited in the application * see the whole document, esp. p.124 l. col. 2.par. - r.col. 1.par *	1-14,20, 21
A	LINCOLN C. ET AL: "A knotted1-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants." PLANT CELL. (1994 DEC) 6 (12) 1859-76. JOURNAL CODE: BJU. ISSN: 1040-4651.. XP002075916 see the whole document	1-14,20, 21
A	AOYAMA T. ET AL.: "Ectopic expression of the Arabidopsis transcriptional activator Athb-1 alters leaf cell fat in tobacco" THE PLANT CELL, vol. 7, no. 11, November 1995, pages 1773-1785, XP002023729 see the whole document	1-14,20, 21
A	CHUCK G ET AL: "KNAT1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis." PLANT CELL. (1996 AUG) 8 (8) 1277-89. JOURNAL CODE: BJU. ISSN: 1040-4651.. XP002075917 see the whole document	1-14,20, 21
A	WO 96 14414 A (JOHN INNES CENTRE ;COUPLAND GEORGE MICHAEL (GB); PUTTERILL JOANNA) 17 May 1996 cited in the application see the whole document	15-19
A	WO 97 10339 A (JOHN INNES CENTRE ;BRADLEY DESMOND JOSEPH (GB); CARPENTER ROSEMARY) 20 March 1997 * see the whole document, esp. p.17 ff. *	15-19

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1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROVENIERS M (REPRINT) ET AL: "The Arabidopsis homeobox gene ATH1 and floral transition" DEVELOPMENTAL BIOLOGY, (15 JUN 1997) VOL. 186, NO. 2, PP. A49-A49. PUBLISHER: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0012-1606., XP002075918 see abstract	1-24

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9614414 A	17-05-1996	AU 3809795 A	31-05-1996
		CN 1171817 A	28-01-1998
		EP 0789765 A	20-08-1997
WO 9710339 A	20-03-1997	AU 6939596 A	01-04-1997
		EP 0852622 A	15-07-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
TR/RM/97058W		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB98/00821	14/05/1998	14/05/1997
International Patent Classification (IPC) or national classification and IPC		
C12N15/29		
Applicant		
D.J. VAN DER HAVE B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability, citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/12/1998	
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax. (+49-89) 2399-4465	Authorized officer Kalsner, I Telephone No. (+49 89) 2399 8708

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB98/00821

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-35 as originally filed

Claims, No.:

1-17 as received on 16/07/1999 with letter of 05/07/1999

Drawings, sheets:

1/9-9/9 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB98/00821

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9, 11-17
	No:	Claims	10
Inventive step (IS)	Yes:	Claims	1-4, 6, 7, 13-17
	No:	Claims	5, 8-12
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/IB98/00821

Ad Section IV: Lack of unity of invention

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.

Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same special technical features, special technical features being such features that define a contribution over which each of the claimed inventions, considered as a whole, makes over the prior art.

In the present application the following inventions have been identified:

1. Claims 1-4: relating to a process for modifying flowering in plants

2. Claims 5-12 : relating to a plant gene construct comprising a DNA sequence for an ATH1 gene product, a transformed plant cell containing said construct and a plant comprising said plant cell

3. Claims 13, 14: relating to a process for inhibiting over-expression of ATH1 in plants

4. Claims 15-17: relating to a plant in which the shade avoidance response is inhibited and a process for producing such plant

The technical relationship between the subject-matter of these groups of claims can only be seen to be the gene encoding ATH1 protein. Since this gene is known in the state of the art (D1) the claims are no longer linked by a common inventive concept referred to above. The presently claimed subject-matter, thus, falls apart in the above groups of inventions which are not unitarian.

As the examination of the present application could be carried out without undue effort, the IPEA chose, according to Rule 68.1 PCT, not to invite the applicant to restrict or pay additional examination fees.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/IB98/00821

Ad Section V: Reasoned statement with regard to novelty, inventive step or industrial applicability

1) **Amendments**

The amendments filed with the letter of 5 July 1999 are formally allowable under Art. 34(2)(b) PCT.

2) **Documents**

D1...Quaedvlieg et al. (1995) The Plant Cell 7: 117-129
D2...WO-A-9614414
D3...WO-A-9710339

D1 describes the characterisation of the homeobox gene ATH1 of Arabidopsis. In additional experiments the coding region of the ATH1 gene with the leader sequence was put under the control of a cauliflower mosaic virus 35S promoter and introduced in Arabidopsis (p. 124, left col., line 31 - right col., line 4).

3) **Novelty** (Inventions 1 and 2)

3.1) **Claim 10** does not meet the requirements of Art. 33(2) PCT in view of D1, as a known product is not rendered novel by producing it by a new process.

3.2) **Claims 1-4** relating to a process for modifying flowering in plants, **claim 5** relating to a plant gene construct comprising a complete or partial DNA sequence coding for an ATH1 gene product under an inducible promoter, **claims 6 and 7** relating to a plant gene construct comprising a complete or partial DNA sequence coding for an ATH1 gene product which product inhibits production of ATH1 protein and **claims 8 and 9** relating to plant cells and a plant containing such construct as well as **claims 11 and 12** meet the requirements of Art. 33(2) PCT.

4) **Inventive step**

4.1) **Claim 5** does not meet the requirements of Art. 33(3) PCT as a plant gene

product comprising a complete or partial DNA sequence coding for an ATH1 gene is known in the prior art (D1). The only difference between the plant gene construct disclosed in D1 and the construct of present claim 5 lies in the promoter which is constitutive in the gene construct of D1 and inducible in the gene construct of claim 5. As various promoters (inducible and constitutive) are well known in the prior art, a plant gene construct that differs from known gene constructs only by the presence of an inducible instead of a constitutive promoter cannot be regarded to involve an inventive step.

Claims 8-12 do not meet the requirements of Art. 33(3) PCT as the provision of plant cells or a plant containing a construct which is not inventive is considered obvious.

4.2) **Claims 1-4**, which are directed to a process for modifying flowering in plants which comprises transforming the plants with a construct comprising a complete or partial DNA sequence coding for an ATH1 gene product, meet the requirements of Art. 33(3) PCT.

Even though processes for modifying flowering in plants have been described in the prior art (e.g. D2, D3) these documents do not disclose or suggest that the gene encoding ATH1 protein could be used for modifying flowering in plants.

Claims 6 and 7 are considered to meet the requirements of Art. 33(3) PCT as a plant gene construct comprising a complete or partial DNA sequence which product inhibits the production of ATH1 protein has not been disclosed nor suggested in the available prior art.

5) **Novelty and inventive step** (Inventions 3 and 4)

Claims 13 and 14 meet the requirements of Art. 33(2)(3) PCT as a process for inhibiting over-expression of ATH1 in a plant which had been transformed in order to over-express ATH1 protein has not been disclosed or suggested in any of the available prior art. Note, however, objections regarding clarity in Section V!

Claims 15-17 which refer to a plant in which the shade avoidance response is

inhibited by inhibition of the formation of ATH1 protein and a process for producing such a plant meet the requirements of Art. 33(2)(3) PCT as the subject-matter of these claims is not disclosed nor suggested in the available prior art.

6) Priority

The validity of the priority date of the present application has not been checked. If, however, the claimed priority is not valid, the documents cited in the International Search Report as "P" (Proveniers et al., 1997) would have to be considered for assessment of novelty and inventive step of the claims which do not enjoy priority.

Ad Section VIII: Certain observations on the international application

Claim 13 does not meet the requirements of Art. 6 PCT for the following reason:

Claim 13 relates to a process for inhibiting over-expression of ATH1 in plants claimed in any of claims 9-12. Claims 9 and 10, however, refer back to claims 1-3, 6, and 7 (among others) which include gene constructs which are designed to inhibit the production of ATH1 in plants. Thus the dependencies of claims 13 and 14 are not considered clear.

WE CLAIM:

1. A process for modifying flowering in plants which comprises transforming the plants with a construct comprising a complete or partial DNA sequence coding for an ATH1 gene product under the control of a promoter functional in plants.

2. A process as claimed in claim 1 whereby the flowering process in plants is promoted by transforming the plants using a construct that inhibits the production of ATH1 protein.

3. A process as claimed in claim 2 in which the construct is adapted to express RNA antisense to RNA produced by the ATH1 gene.

4. A process as claimed in claim 1 whereby the flowering process in plants is retarded by transforming the plants using a construct that promotes the production of recombinant ATH1 protein.

5. A plant gene construct useful in the process of claim 1 which comprises a complete or partial DNA sequence coding for an ATH1 gene product under the control of an inducible promoter functional in plants.

6. A plant gene construct useful in the process of claim 2 which comprises a complete or partial DNA sequence coding for an ATH1 gene product under the control of an promoter functional in plants, which product inhibits the production of ATH1 protein.

7. A plant gene construct as claimed in claim 6 in which the gene product is antisense RNA.

AMENDED SHEET

8. Transformed plant cells containing constructs claimed in any of claims 5-7.

9. A plant containing plant cells claimed in claim 8.

10. A genetically modified plant produced by the process claimed in any of claims 1-4.

11. A plant claimed in claims 9 or 10 which is a crop plant.

12. A plant as claimed in claim 11 which is rice, maize, wheat, barley, oats, rye, lettuce, endive, oilseed rape (canola), sugar beet, sunflower, soya or sorghum.

13. A process for inhibiting over-expression of ATH1 in plants claimed in any of claims 9-12 which comprises treating the plants with a gibberellin.

14. A process as claimed in claim 13 in which the gibberellin is A3 or A4/A7.

15. A plant in which the shade avoidance response is inhibited by the action of a transgene coding for a gene product that inhibits the formation of ATH1 protein.

16. A plant as claimed in claim 16 in which the transgene is a construct adapted to express antisense RNA.

17. A process for producing a plant as claimed in claims 15 or 16 which comprises:

transforming cells of a plant showing a shade avoidance response with a plant gene construct claimed in claim 7; and regenerating plants from said transformed plant cells.

twr 5 July 1999

AMENDED SHEET

WE CLAIM:

1. A plant gene construct comprising a complete or partial DNA sequence coding for an ATH1 gene product under the control of a promoter functional in plants.

2. A plant gene construct as claimed in claim 1 in which the promoter is heterologous.

3. A plant gene construct as claimed in claim 2 in which the promoter is constitutive.

4. A plant gene construct as claimed in claim 2 in which the promoter is inducible.

5. A plant gene construct as claimed in any of claims 1-4 in which the complete or partial DNA sequence is homologous with the DNA sequence shown in Figure 1.

6. A plant gene construct as claimed in any of claims 1-5 which is adapted to express RNA antisense to RNA produced by the ATH1 gene.

7. A plant gene construct as claimed in any of claims 1-5 which is adapted to express RNA homologous to RNA produced by the ATH1 gene.

8. A plant cell transformed with a DNA construct claimed in any of claims 1-7.

9. A plant cell as claimed in claim 8 adapted to express RNA that produces recombinant ATH1 protein in the cell.

10. A plant cell as claimed in claim 8 adapted to express RNA that inhibits the production of ATH1 protein in the cell.

11. A plant comprising transformed plant cells as claimed in any of claims 8-10.

12. A plant as claimed in claim 11 which is a crop plant.

13. A plant as claimed in claim 12 which is rice, maize, wheat, barley, oats, rye, lettuce, endive, oilseed rape (canola), sugar beet, sunflower, soya or sorghum.

14. A plant as claimed in either of claims 12 or 13 adapted to produce recombinant ATH1 protein.

15. A process for modifying flowering in plants which comprises transforming the plants with a construct as claimed in any of claims 1-5.

16. A process as claimed in claim 15 whereby the flowering process in plants is promoted by transforming the plants with a construct claimed in either of claims 6 or 7 that inhibits the production of ATH1 protein.

17. A process as claimed in claim 15 whereby the flowering process in plants is retarded by transforming the plants with a construct claimed in claim 7 that promotes the production of recombinant ATH1 protein.

18. A process for inhibiting over-expression of ATH1 in plants claimed in claim 14 which comprises treating the plants with a gibberellin.

19. A process as claimed in claim 18 in which the gibberellin is A3 or A4/A7.

20. A plant DNA construct comprising the ATH1 promoter linked to heterologous DNA so as to cause transcription thereof in plant cells.

21. Plant cells transformed with a construct claimed in claim 20.

22. A plant lacking a shade avoidance response comprising: a plant transformed with a transgene wherein said transgene induces a shade avoidance response in said transformed plant.

23. A plant according to claim 22 wherein said transformed plant is formed from a wildtype plant which has a shade avoidance response.

24. A method of producing a transgenic plant that lacks the shade avoidance response of a wildtype plant, comprising:
forming a construct having a complete or partial DNA sequence coding for an ATH1 gene product;
transforming said wildtype plant material with said construct; and
forming plants therefrom.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* TR/RM/97058WO

Box No. I TITLE OF INVENTION

Plant Gene Constructs and Their USE

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

D.J. van der Have B.V.
Dijkwelsestraat 70
4420 AA KAPELLE
The Netherlands

☐ This person is also inventor.

Telephone No. 31 113 347 911

Facsimile No. 31 113 330 110

Teleprinter No.

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

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The Netherlands

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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Facsimile No. 44 171 253 1214

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

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WEISBEEK, Peter
Baarnseweg 49
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The Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of:
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

PROVENIERS, Marcel
Kampereiland 11
3524 CZ UTRECHT
The Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of:
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
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- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

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This person is applicant for the purposes of:
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
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State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
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Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

	Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1)	UK	14 May 1997 14.05.97	9709789.3	
item (2)	UK	30 December 1997 30.12.97	9727458.3	
item (3)				

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): _____

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Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EP

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	: 4	sheets
2. description	: 35	sheets
3. claims	: 3	sheets
4. abstract	: 1	sheets
5. drawings	: 9	sheets
Total	: 52	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
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6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

WEITZEL, David Stanley
Agent

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1. Date of actual receipt of the purported international application:
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PCT/IB98/0082

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
27 September 1999 (27.09.99)

Applicant's or agent's file reference
TR/RM/97058W J15446

IMPORTANT NOTIFICATION

International application No.
PCT/IB98/00821

International filing date (day/month/year)
14 May 1998 (14.05.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

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Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
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NL-4420 AA Kapelle
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

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0028

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00821

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N15/11 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAUL R. ROBSON ET AL: "Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene." NATURE BIOTECHNOLOGY, (1996) 14/8 (995-998). ISSN: 0733-222X CODEN: NABIF, XP002075914 cited in the application	22,23
A	see the whole document	24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1998	21/09/1998

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00821

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	QUAEDVLIEG N ET AL: "The homeobox gene ATH1 of Arabidopsis is derepressed in the photomorphogenic mutants cop1 and det1." PLANT CELL, (1995 JAN) 7 (1) 117-29. JOURNAL CODE: BJU. ISSN: 1040-4651., XP002075915 cited in the application * see the whole document, esp. p.124 l. col. 2.par. - r.col. 1.par *	1-14,20, 21
A	LINCOLN C. ET AL: "A knotted1-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants." PLANT CELL, (1994 DEC) 6 (12) 1859-76. JOURNAL CODE: BJU. ISSN: 1040-4651., XP002075916 see the whole document	1-14,20, 21
A	AOYAMA T. ET AL.: "Ectopic expression of the Arabidopsis transcriptional activator Athb-1 alters leaf cell fat in tobacco" THE PLANT CELL, vol. 7, no. 11, November 1995, pages 1773-1785, XP002023729 see the whole document	1-14,20, 21
A	CHUCK G ET AL: "KNAT1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis." PLANT CELL, (1996 AUG) 8 (8) 1277-89. JOURNAL CODE: BJU. ISSN: 1040-4651., XP002075917 see the whole document	1-14,20, 21
A	WO 96 14414 A (JOHN INNES CENTRE ;COUPLAND GEORGE MICHAEL (GB); PUTTERILL JOANNA) 17 May 1996 cited in the application see the whole document	15-19
A	WO 97 10339 A (JOHN INNES CENTRE ;BRADLEY DESMOND JOSEPH (GB); CARPENTER ROSEMARY) 20 March 1997 * see the whole document, esp. p.17 ff. *	15-19

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00821

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citat on of document, with indicat on, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROVENIERS M (REPRINT) ET AL: "The Arabidopsis homeobox gene ATH1 and floral transition" DEVELOPMENTAL BIOLOGY, (15 JUN 1997) VOL. 186, NO. 2, PP. A49-A49. PUBLISHER: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0012-1606., XP002075918 see abstract	1-24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 98/00821

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9614414	A	17-05-1996	AU	3809795 A	31-05-1996
			CN	1171817 A	28-01-1998
			EP	0789765 A	20-08-1997
WO 9710339	A	20-03-1997	AU	6939596 A	01-04-1997
			EP	0852622 A	15-07-1998

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/29, 15/82, 15/11, 5/10, A01H 5/00

A1

(11) International Publication Number: **WO 98/51800**

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(21) International Application Number: PCT/IB98/00821

(22) International Filing Date: 14 May 1998 (14.05.98)

(30) Priority Data:

9709789.3	14 May 1997 (14.05.97)	GB
9727458.3	30 December 1997 (30.12.97)	GB

(71) Applicant *(for all designated States except US)*: D.J. VAN DER HAVE B.V. [NL/NL]; Dijkwelsestraat 70, NL-4420 AA Kapelle (NL).

(72) Inventors; and

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(74) Agent: ROBERTS, T., W.; Batchellor, Kirk & Co., 2 Pear Tree Court, Farringdon Road, London EC1R 0DS (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PLANT GENE CONSTRUCTS AND THEIR USE

(57) Abstract

A plant gene construct is disclosed comprising a complete or partial DNA sequence coding for an ATHi gene product under the control of a promoter functional in plants. The promoter is preferably heterologous. Plant cells are transformed with such a plant gene construct, and plants comprising such cells have modified flowering properties. There is further described a process for modifying the flowering process in plants by transforming plants with a construct according to the invention.

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PLANT GENE CONSTRUCTS AND THEIR USE

The present invention relates to novel plant gene constructs, and to their use in controlling the flowering of plants. It further relates to plants containing such constructs.

Plants differ from animals. The adult plant body is formed post-embryonically by the continuous activity of the shoot and root apical meristems. The shoot apical meristem is established during plant embryogenesis and together with cotyledons, hypocotyl, embryonic root and root meristem makes up the basic body plan.

The shoot apical meristem starts as a cluster of about one hundred cells and is the source of the whole aboveground portion of the plant. During the vegetative phase of plant development this meristem gives rise to (a rosette of) leaves, stem, and quiescent axillary meristems. This is followed by the formation of secondary inflorescences, cauline leaves and determinate floral meristems after floral induction. Flowering involves complex interactions of gene products that regulate a switch in shoot meristem identity. Factors determining the expression levels of these genes are genotype and environmental stimuli, such as photoperiod, temperature and light quality. How the transition is affected by these stimuli is still largely unknown.

One of the most important events in the plant life cycle is the decision to enter the reproductive phase. A wide range of environmental and endogenous signals controls this transition of the vegetative phase into the reproductive phase. Important signals are day length, temperature (vernalization), nutrient and water availability and

several phytohormones esp. gibberellin (GA). These signals induce a shift in vegetative apical meristem identity, named the floral transition, and this transition establishes the inflorescence meristem. Whereas the product of the vegetative apical meristem are leaf primordia, the inflorescence meristem produces primordia that differentiate into secondary inflorescences during early generative development and into flowers later in this stage. In plant breeding research, control of this process is a most important goal for a variety of crops. This is especially true for rosette plants like lettuce, spinach and sugar beet, which show rapid stem elongation (bolting) following the floral transition, and this makes the crop useless.

The transition from vegetative to reproductive growth is a critical developmental event, and because it is the first step of sexual reproduction it is of great importance in agriculture, horticulture, and plant breeding. Farmers may wish to advance or retard the time of flowering, or prevent it altogether: for example to prevent 'bolting' in e.g. lettuces or sugar beet. A better understanding of the molecular biology of plant flowering will allow it to be controlled or influenced in a number of ways, giving important practical benefits to agriculture.

In PCT Publication WO96/14414, use of the *Constans* (*CO*) gene to modify flowering mechanisms in plants is disclosed.

The present invention proposes a way of influencing a plant's transition from vegetative to reproductive growth, by providing transformed plants in which the transition is delayed, or brought forward, by expression of specific transgenes influencing this process. Such genes may be constitutively expressed, or expressed only in response to

an external stimulus, for example environmental or chemical.

ATH1 is an *Arabidopsis thaliana* homeobox gene. It is described by Quaedvlieg et al., in Plant Cell 7, 117-129, 1995 (herein incorporated by reference): its DNA sequence is given in Figure 1 of that paper. It was isolated from a light-induced transcription factor collection. It is expressed in young seedlings and flowers. ATH1 mRNA levels in etiolated seedlings are strongly light-dependent (phytochrome) and are also light-adaptive.

We have now established that the protein product of ATH1 is involved in the developmental switch from vegetative to generative growth. As a result of ATH1::GUS studies and initial 35S::ATH1 studies, we have deduced that ATH1 has a function in the transition of the vegetative apical meristem to an inflorescence meristem. Specifically, ATH1 acts as an anti-gibberellin, by repressing GA synthesis or possibly the GA response pathway: Example 6 illustrates this.

Our studies on ATH1::GUS constructs have revealed that in young, light-grown seedlings ATH1 is expressed in all three layers of the shoot apical meristem and leaf primordia. In young, still developing leaves ATH1 is expressed in vascular tissue. This expression disappears in developed leaves. Remarkably, ATH1 meristem expression is restricted to the vegetative phase of development. As soon as *Arabidopsis* starts flowering (vegetative to generative transition) and the shoot apical meristem has become an inflorescence meristem, ATH1 expression in the meristem is downregulated. During the inflorescence phase ATH1 is at a low level expressed in developing vascular tissue of the

stem. Later in plant development, when flowers arise, ATH1 is expressed in different parts of the young flower (receptacle, sepals and vascular tissue of stamen). Our hypothesis that ATH1 is involved in controlling the phase transition from vegetative to generative growth is further corroborated by the flowering time phenotypes of ATH1 sense and antisense over-expressors. Plants ectopically overexpressing antisense ATH1 show an early-flowering phenotype: conversely, most plants carrying a sense ATH1 overexpression construct are late flowering. A small proportion of the plants carrying the overexpression construct are, due to ATH1 reduction by co-suppression, early flowering, like the antisense ATH1 over-expressors, and the phenotype of these plants resembles that of the terminal flower mutant(Shannon and Meeks-Wagner, 1991) and the phenotypes of LEAFY- (Weigel and Nilsson, 1995), APETALA 1- (Mandel and Yanofsky, 1995) and CONSTANS (Putteril *et al.*, 1995)over-expressors. Based on these results, combined with the ATH1::GUS data, we deduce that ATH1 is involved in controlling the phase transition from vegetative to generative growth in *Arabidopsis thaliana*, and probably is a flowering time gene.

In consequence, this transition may be promoted by inhibiting the expression of the ATH1 gene: or retarded or prevented by promoting such expression.

Accordingly, the present invention provides a plant gene construct comprising a complete or partial DNA sequence coding for an ATH1 gene product under the control of a promoter functional in plants. The promoter is preferably heterologous. The invention further comprises plant cells transformed with a such a plant gene construct, and plants comprising such cells having modified flowering properties. The invention further comprises a process for modifying the

flowering process in plants by transforming plants with a construct according to the invention.

The use of gene sequences to inhibit or promote gene expression is quite well understood. A complete gene sequence, under the control of a promoter that operates effectively in the plant, will generally overexpress the gene product, leading to an amplification of the effect of the protein so produced. Sometimes the gene product is reduced: this phenomenon is termed "co-suppression". Reduction of the gene product is also generally obtained by using a dominant negative mutation, or by reversing the orientation of the gene sequence with respect to the promoter so that it produces "antisense" messenger RNA.

A DNA construct according to the invention may be an "antisense" construct generating "antisense" RNA or a "sense" construct (encoding at least part of the functional protein) generating "sense" RNA. "Antisense RNA" is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA: complementary in the sense that each base (or the majority of bases) in the antisense sequence (read in the 3' to 5' sense) is capable of pairing with the corresponding base (G with C, A with U) in the mRNA sequence read in the 5' to 3' sense. Such antisense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged to generate a transcript with at least part of its sequence complementary to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). "Sense RNA" is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence. Such sense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged in the normal orientation so as to generate a transcript

with a sequence identical to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). Suitable sense constructs may be used to inhibit gene expression (as described in International Patent Publication WO91/08299) or a sense construct encoding and expressing the functional protein may be transformed into the plant to over-express the protein.

DNA constructs according to the invention may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence - it may be as long as the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. The preparation of such constructs is described in more detail below.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used. The isolation of suitable ATH1 sequences from *Arabidopsis* is described in Quaedvlieg et al., above: similar methods may be used to isolate ATH1 sequences from other plants. These may have greater or lesser degrees of homology with ATH1 sequences from *Arabidopsis*. Sequences coding for the whole, or substantially the whole, of the protein may thus be obtained. Suitable lengths of this DNA sequences may be cut out for use by means of restriction enzymes. When using genomic DNA as the source of a partial base sequence for transcription it is possible to use either intron or exon regions or a combination of both.

To obtain constructs suitable for modifying expression of ATH1 in plant cells, the cDNA sequence as found in the protein cDNA or the gene sequence as found in the chromosome of the plant may be used. Recombinant DNA constructs may be made using standard techniques. For example, the DNA sequence for transcription may be obtained by treating a vector containing said sequence with restriction enzymes to cut out the appropriate segment. The DNA sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to give suitable restriction sites at each end. The DNA sequence is then cloned into a vector containing upstream promoter and downstream terminator sequences. If antisense DNA is required, the cloning is carried out so that the cut DNA sequence is inverted with respect to its orientation in the strand from which it was cut.

In a construct expressing antisense RNA, the strand that was formerly the template strand becomes the coding strand, and vice versa. The construct will thus encode RNA in a base sequence which is complementary to part or all of the sequence of the protein mRNA. Thus the two RNA strands are complementary not only in their base sequence but also in their orientations (5' to 3').

In a construct expressing sense RNA, the template and coding strands retain the assignments and orientations of the original plant gene. Constructs expressing sense RNA encode RNA with a base sequence which is homologous to part or all of the sequence of the mRNA. In constructs which express the functional protein, the whole of the coding region of the gene is linked to

transcriptional control sequences capable of expression in plants.

For example, constructs according to the present invention may be made as follows. A suitable vector containing the desired base sequence for transcription (such as the pATH1 cDNA clone) is treated with restriction enzymes to cut the sequence out. The DNA strand so obtained is cloned (if desired, in reverse orientation) into a second vector containing the desired promoter sequence and the desired terminator sequence. Suitable promoters include the 35S cauliflower mosaic virus promoter and the tomato polygalacturonase gene promoter sequence (Bird et al, 1988, Plant Molecular Biology, 11:651-662) or other developmentally regulated plant promoters. Suitable terminator sequences include that of the Agrobacterium tumefaciens nopaline synthase gene (the nos 3' end).

In a DNA construct according to the invention, the transcriptional initiation region may be derived from any plant-operative promoter. The transcriptional initiation region may be positioned for transcription of a DNA sequence encoding RNA which is complementary to a substantial run of bases in a mRNA encoding the ATH1 protein (making the DNA construct a full or partial antisense construct).

The transcriptional initiation region (or promoter) operative in plants may be a constitutive promoter (such as the 35S cauliflower mosaic virus promoter) or an inducible or developmentally regulated promoter, as circumstances require. For example, it may be desirable to modify protein activity at certain stages of the plant's development. Use of a constitutive promoter

will tend to affect protein levels and functions in all parts of the plant, while use of a tissue-specific promoter allows more selective control of gene expression and affected functions. Thus the antisense or sense RNA is only produced in the organ in which its action is required.

The DNA constructs of the invention may be inserted into plants to regulate the expression of the ATH1 gene resulting in modification of plant characteristics (in particular flowering). Depending on the nature of the construct, the production of the ATH1 gene product may be increased, or reduced, either throughout or at particular stages in the life of the plant. Generally, as would be expected, production of the protein is enhanced only by constructs which express RNA homologous to the substantially complete endogenous protein mRNAs. Full-length sense constructs may also inhibit protein expression. Constructs containing an incomplete DNA sequence shorter than that corresponding to the complete gene generally inhibit the expression of the gene and production of the proteins, whether they are arranged to express sense or antisense RNA.

A DNA construct of the invention is transformed into a target plant cell. The target plant cell may be part of a whole plant or may be an isolated cell or part of a tissue which may be regenerated into a whole plant. The target plant cell may be selected from any monocotyledonous or dicotyledonous plant species. Plants may be derived from the transformed plant cell by regeneration of transformants and by production of successive generations of the transformants' progeny.

Constructs according to the invention may be used to transform any plant using any suitable

transformation technique to make plants according to the invention. Both monocotyledonous and dicotyledonous plant cells may be transformed in various ways known to the art. In many cases such plant cells (particularly when they are cells of dicotyledonous plants) may be cultured to regenerate whole plants which subsequently reproduce to give successive generations of genetically modified plants. Any suitable method of plant transformation may be used. For example, dicotyledonous plants such as tomato and melon may be transformed by *Agrobacterium* Ti plasmid technology, such as described by Bevan (1984, Nucleic Acid Research, 12:8711-8721) or Fillatti et al (Biotechnology, July 1987, 5:726-730). Such transformed plants may be reproduced sexually, or by cell or tissue culture. Monocots may be transformed by use of the gene gun. Other methods for plant transformation include microinjection and electroporation.

Examples of genetically modified plants according to the present invention include cereals, for example rice and maize, wheat, barley, oats and rye. Other important seed products are oilseed rape (canola), sugar beet, sunflower, soya and sorghum. Most crops are grown annually from seed and the production of seed of any kind depends upon the ability of the plant to flower, to be pollinated and to set seed. In horticulture, control of the timing of flowering is important. Horticultural plants whose flowering may be controlled include lettuce, endive and vegetable brassicas including cabbage, broccoli and cauliflower, and carnations and geraniums.

The main characteristics of modified plants according to the invention are early or delayed flowering. Genotypes in which production of the ATH1 protein is inhibited generally flower early: genotypes in which it is stimulated flower

late. Other effects on plant phenotype may also be observed, e.g. dwarf habit, for example in tobacco.

Control of the time of flowering may be useful for several reasons. For example, flowering may be controlled to provide flowers or fruit at the time most appropriate for marketing. In hybrid production, flowering of male and female parents may be co-ordinated. It is most convenient to do this by the use of inducible gene promoters, responsive to external stimuli, for example application of chemicals. An example of such a promoter is the maize glutathione-S-transferase isoform II gene promoter, activated by application of a known herbicide safening agent (WO93/01294 to ICI).

Bolting control may be economically important in several crop species. For example, in sugarbeet, producing varieties which have a reduced tendency to bolt after cold treatment would be of great use. Processing factories could spread their activities over a longer period of time, with significant savings in overheads. Bolting-resistant varieties could be sown very early in the season (February) or even the year before in autumn (provided winter frost was not a problem). Further, varieties in which bolting is increased may be bred faster: crossings may be carried out annually instead of biannually as at present.

Early flowering sunflower would have an extended geographical range. It could be grown further north (north of Paris), and possibly in drier regions, e.g. parts of Spain, avoiding periods of drought later in summer.

In vegetables, bolting may be controlled in for example lettuce and endive. This would allow growing the crop more

easily during summer. Existing varieties tend to bolt rather rapidly under summer conditions. In grasses, reduced (or no) bolting is beneficial for fodder types (improved feed quality) and amenity types (better quality lawns).

It will on occasion be of advantage to time the expression of transgenes to stop when flowering starts, or suppress naturally-occurring genes until flowering starts. This may be done using the ATH1 promoter to control expression of a transgene, or transcription of DNA homologous to a natural gene. Accordingly it is a further separate feature of the invention to provide a DNA construct comprising the ATH1 promoter linked to heterologous DNA so as to cause transcription thereof in plant cells: and plant cells transformed with such DNA constructs.

ATH1 is expressed in the vegetative apical meristem, and downregulation of this expression coincides with floral transition. Forced constitutive expression of ATH1 results in a dramatic repression of floral transition both in *Arabidopsis* and tobacco: thus, in the case of *Arabidopsis* bolting is postponed. Conversely, repression of ATH1 results in an early flowering phenotype. Our results suggest that ATH1 exerts its function through modulation of GA biosynthesis or responsiveness. We expect the ATH1 gene to be the basis of a particularly useful bolting control system.

Day length and floral transition

The floral transition has been particularly well investigated in *Arabidopsis thaliana*. This species has become the model system for studying floral transition: at the genetic level through the isolation of flowering-time mutants; and at the molecular level through cloning of

genes whose products participate in the control of floral transition. *Arabidopsis* is a typical rosette plant in which the vegetative leaves are closely spaced due to reduced internode elongation. Upon floral transition the newly formed internodes rapidly elongate ('bolting'). In most *Arabidopsis* ecotypes day length is of major importance in determining floral transition. *Arabidopsis* is a facultative long day (LD) plant, which means that floral transition is hastened by long days (16 hours light/8hours dark cycle), but there is no obligate requirement for it. Under long day (LD) conditions floral transition is rapidly initiated and only a few rosette leaves are formed (~7 leaves, 16-20 days for the Col-0 ecotype). When grown under short days (SD), e.g. 8 hrs light/16 hrs dark, floral transition takes much longer (~60 days) and a full leafy rosette is formed which can have in excess of ~30 rosette leaves (Col-0 ecotype).

Gibberellic acid (GA) and floral transition

It has been known for a long time that GA treatment promotes floral transition in a variety of plant species. Most species in which applied GA can induce flowering are long-day or cold-requiring plants, and many of these normally grow as rosettes under non-inductive conditions. Moreover, several experiments suggest that endogenous GA levels are involved in controlling floral transition: conditions that induce floral transitions can exert their effect through elevation of endogenous GA levels probably at or near the apical meristem.

Arabidopsis mutants defective in GA biosynthesis (GA series) or insensitive to this hormone (GAI series) show a late flowering phenotype under non-inductive conditions and moreover, the severe GA1-3 mutant is also late flowering under inductive conditions (Wilson et al., 1992).

Involvement of GA in ATH1 control of floral transition

We tested whether exogenous GA can overcome the inhibitory effect of constitutive ATH1 expression in tobacco. Most remarkably, GA spraying was able to 'rescue' the late flowering phenotype in constitutive ATH1 expressers in tobacco. An involvement of GA was also indicated by the reduced internode elongation phenotype in the tobacco ATH1 expressers. These findings suggest that ATH1 functions as a repressor of GA biosynthesis or, alternatively, of GA responsiveness. The dominant effect of ATH1 over-expression on floral transition in combination with the reversion of this effect by exogenously added GA suggests several uses in a variety of crops. This is especially interesting since deregulated expression of ATH1 does not lead to pleiotropic phenotypes and reversion of the overexpression phenotype is complete. Complete rescue means that there will be no problems regarding reproduction or multiplication of ATH1-transformants: thus maintaining the transgenic lines, which can be a serious problem with flowering mutants, is straitforward. Using the GA switch, plants can be reversed to wild-type development at any moment, plants flower normally, and there is a normal seed set.

Accordingly, it is a further feature of the invention to inhibit over-expression of ATH1 in plants genetically modified according to the invention by treating the plants with a gibberellin, for example gibberellin A3 or A4/A7.

Increase of harvest index

When plants grow in close proximity, shade-avoidance syndrome, in which plants react to far-red radiation

reflected from neighbors, is manifested. This most obviously results in a rapid and dramatic increase in the extension growth of stems and petioles at the expense of leaf growth, storage organ production, and reproductive development. It is known that by overexpression of phyA genes in tobacco the shade-avoidance response can be overcome, resulting in an increased harvest index (Robson et al., 1996). Harvest index is expressed as leaf biomass as a proportion of total biomass. Overexpression of ATH1 in tobacco causes a reduction in stem growth, while leaf growth and number stay unaffected or even increase compared to wild type. As ATH1 overexpression phenotypes and phyA overexpression phenotypes are similar, this suggests using ATH1 overexpression to increase harvest index in crops. The invention will be further described with reference to the following Examples and Experiments, which illustrate certain aspects of our invention: and with respect to the drawings, in which:

Figure 1 gives the DNA sequence of ATH1 cDNA;

Figure 2 is a diagram of the plasmid pWP90;

Figure 3 is a diagram of the plasmid pMOG23;

Figure 4 is a diagram of the plasmid pVDH275;

Figure 5 is a bar graph showing the dwarfing caused by constitutive expression of ATH1 in 90 days old tobacco plants;

Figure 6 is a graph showing the effect of gibberellin treatment on the height of tobacco plants overexpressing ATH1;

Figure 7 is a bar graph showing flowering time (in terms of number of rosette leaves formed) for under- and over-expressors of ATH1 in comparison with wild-type *Arabidopsis* (C24 ecotype).

General Methods

Plant material and plant growth conditions

The wild-type genotypes used were *Arabidopsis thaliana* Columbia and C24. The ATH1 gene is located on chromosome 4, between the RFLP markers mi431 (96.9 cM) and G6455 (97.9 cM). *Arabidopsis thaliana* Columbia was used in plant transformation experiments using the vacuum infiltration protocol, while *Arabidopsis thaliana* C24 was used in plant transformation experiments using the root transformation protocol.

Plants were grown in a growth chamber under fluorescent light with a photoperiod of 16 hours followed by an 8 hours dark period at a continuous temperature of 22°C.

To measure flowering time seeds were imbibed and placed at 4°C for 4 days to break dormancy and were then sown on soil. Germinating seedlings were usually covered with propagator lids for the first 1-2 weeks to prevent dehydration.

Transformation of *Arabidopsis* plants

Binary constructs containing chimeric ATH1-GUS genes and 35S-antisense ATH1 genes were transformed into *Arabidopsis thaliana* ecotype C24 using the *Agrobacterium tumefaciens*-mediated root transformation method of Valvekens et al. (1988). Transformants were selected on medium containing 50 mg/l kanamycin.

Binary constructs containing chimeric 35S-ATH1 genes were transformed into *Arabidopsis thaliana* ecotype Columbia using the vacuum infiltration protocol (Bent et al. (1994); Bechtold et al. (1993)) with some modifications. Plants

were grown separately in 5.5 cm pots. Plants were transformed after appearance of the first siliques on the secondary bolts.

900 ml cultures of *Agrobacterium tumefaciens* containing the appropriate construct were grown the night before the day of infiltration, cells were harvested by centrifugation and resuspended in an equal volume of infiltration medium, containing 2% instead of 5% sucrose. Plants were infiltrated by submerging entire rosettes and bolts for 10 minutes under a vacuum pressure of 100mm Hg.

Transformant seeds were selected on medium containing 50 mg/l kanamycin.

EXAMPLE 1

ATH1 expression analysis

Total RNA isolation

Total RNA from plants was isolated according to De Vries et al. (1988) with some minor modifications: (1) plant tissue was ground in liquid nitrogen in the presence of half the volume of phenol/extraction buffer and heated to 65°C in a water-bath and (2) the RNA was ethanol/Na-acetate precipitated before and after LiCl precipitation.

RNAase protection analysis

The HindIII-XhoI fragment of phagemid ATH1 was cloned into pBluescriptSK(-) (Stratagene) and digested with HindIII to produce a T7 RNA polymerase template. The ATH1 RNA probe protects a fragment of 140 nt. RNA probe was synthesized by using T7 RNA polymerase (Pharmacia) and buffer as

described by the manufacturer, except that 160 µCi of [-^{32}P]UTP (800 Ci/mmol) was used. RNAase protection was done by using 10 µg of total RNA and 10ug of tRNA according to the protocol described by Sambrook et al. (1989). The digested mixture contained 600 units/ml RNAase T1 (Gibco BRL) and 20 ug/ml RNAase A (Boehringer). RNA:RNA hybrids were analyzed by sequence gel electrophoresis (6% polyacrylamide/ 7M urea) and visualized by autoradiography.

Construction of chimeric ATH1-GUS constructs

A SpeI-NcoI fragment containing approximately 1300 nucleotides of ATH1 promoter sequence was isolated. After filling in the *NcoI* site with Klenow-polymerase, this fragment was inserted into the unique *SmaI/XbaI* sites of the pBi101.1 binary vector which contains the GUS gene (Jefferson et al., 1987), creating a translational fusion between the ATH1 promoter and the GUS gene. The protein encoded by this chimeric gene consists of 42 aa of ATH1 fused to the GUS protein. The binary construct was called tH1.4. tH1.4 was transformed into competent *Agrobacterium tumefaciens* LBA4404 cells (Gelvin and Schilperoort, 1988). *Arabidopsis* lines (ecotype C24) were transformed as described below.

***In situ* localization of GUS activity in transgenic ATH1-GUS *Arabidopsis thaliana* lines**

Seedlings and plant tissues were collected and stained for 1 to 16 hours at 37°C in a solution containing 0.5 mg/ml X-Gluc (Biosynth AG) dissolved in n-dimethyl-formamide, 0,1% Triton X-100, 0.5 mM K4Fe(CN)6.H2O, 0.5 mM K3Fe(CN)6 and 50 mM sodium phosphate buffer, pH 7.2.

Light microscopy

After X-Gluc staining, plant tissues were fixed overnight in a solution containing 1% glutaraldehyde and 4% formaldehyde in 50mM sodium phosphate buffer, pH 7.2. Subsequently seedlings were dehydrated in gradual steps: 10%, 30%, 50%, 70%, 90% and 2x 100% ethanol. Large plant tissues were pre-embedded first in 1% agarose (Sigma). Infiltration and embedding in Technovite 7100 (Kulzer, Hereaus) was performed as instructed by the manufacturer. 4 µm sections were made on a Reichert-Jung 1140 rotary carrying a disposable Adams steel knife. Sections were stained with 0.1% Ruthenium red (Sigma) in distilled water for 2 minutes at room temperature and photographed on a Zeiss Axioskop using Kodak Professional Ektar 25 film.

Seedlings were fixed and dehydrated as above. Technovit 7100 was infiltrated for 1 day. The seedlings were then transferred to a construction of celluloid transparency (Amovis), double-sided tape, transparency, double-sided tape. In the latter three layers a central region was excised to contain the seedling. Subsequently the seedlings were added in Technovit 7100 solution and the central region was covered by another transparency. Upon overnight polymerisation at room temperature a plastic platelet containing the seedling was obtained. In order to section embedded seedlings in the platelet, the celluloid sheet material was removed and the platelet was cut to allow longitudinal sectioning of relevant seedling regions. Sectioning, staining and photographing was performed as described above.

Localization of ATH1 expression

The expression of the ATH1 gene was analyzed using RNA-ase protection analysis (Quaedvlieg et al., 1995). High levels of ATH1 mRNA were detected during early seedling

development (days 2-6) and in flowers of mature *Arabidopsis* plants. The cellular localization of ATH1 gene expression was determined by introduction of the chimeric ATH1-GUS construct tH1.4 in *Arabidopsis thaliana*. Different tissues were stained with X-gluc, and whole mount preparations and tissue sectioning were made to visualize GUS activity (see below).

ATH1 expression during vegetative development

The shoot apex of a 5-day-old light-grown seedling is flat and consists of a two-layered tunica enclosing the subjacent corpus. At this stage, the meristem has initiated the primordia of the first leaf pair (Mischke and Brown, 1965).

In plants transformed with tH1.4, high levels of GUS activity were present in the shoot apex. Sectioning of the shoot apex showed that the high GUS activity is shown in all three layers of the shoot apical meristem and extends through the subapical region, proceeding down to where the vascular strand of the hypocotyl branches into the cotyledons. High levels of GUS activity were also present in the primordia of the first leaf pair.

ATH1 expression during floral transition and inflorescence development

Initially, during the inflorescence phase, the shoot apical meristem gives rise to stem, cauline leaves and secondary inflorescences. As inflorescence development proceeds, the inflorescence meristem produces flower primordia. In plants transformed with tH1.4, GUS activity was downregulated in the inflorescence meristem during the transition phase. There was no GUS activity detectable in the meristem. Low

levels of GUS activity were present in the rib zone. Later when flowers arose, GUS activity was present in different parts of the young flower (receptacle, sepals and vascular tissue of stamen)

EXAMPLE 2

Construction of promoter fusions to the ATH1 open reading frame

The ATH1 cDNA is cloned into the unique EcoRI/XhoI restriction sites of the well-known and commercially available pBluescript SK(-) vector (Stratagene).

2.1. A CaMV 35S promoter fusion to the ATH1 open reading frame

A BamHI/SnaBI fragment containing 1573 nucleotides of ATH1 cDNA sequence (the BamHI site was created by PCR mutagenesis, 35 nucleotides downstream of the translation start) was isolated and inserted into the unique BamHI/SmaI cloning sites of pWP90-vector, which contains a double 35S CaMV promoter and a NOS terminator (see Figure 2), resulting in a transcriptional fusion between the double 35S CaMV promoter and ATH1 cDNA. This construct, called cH1.24, was then cut with SstI/EcoRV restriction enzymes, followed by insertion of the resulting SstI/EcoRV insert in the unique SstI/SmaI restriction sites of binary vector pMOG23 (see Figure 3). The binary construct was called tH1.2. tH1.2 was transformed into competent *Agrobacterium tumefaciens* pGV2260 cells (Caplan *et al.*, 1985) cells. *Arabidopsis* lines (ecotype Col-0) were transformed via vacuum infiltration as described below

2.2 Construction of a CaMV 35S promoter fusion to the antisense ATH1 frame

An EcoRI/SnaBI fragment containing approximately 1830 nucleotides of ATH1 cDNA sequence was isolated and inserted into the unique SmaI/EcoRI cloning sites of pWP90 vector (see Figure 2), resulting in a transcriptional fusion between the double CaMV 35S promoter and the antisense ATH1 frame. The resulting construct was called cH1.22. An EcoRV/SstI insert of cH1.22 was then cloned into the unique SmaI/SacI restriction sites of the binary vector pMOG23 (MOGEN)(see Figure 3). This binary construct, called tH1.1, was transformed into competent *Agrobacterium tumefaciens* LBA4404 cells. *Arabidopsis* lines (ecotype C24) were transformed as described below.

2.3. A heat shock promoter fusion to the ATH1 open reading frame

By PCR mutagenesis, an additional BamHI site was created in pTT19, a vector containing the promoter, leader and 77 nuclectides of coding sequence of the *Arabidopsis thaliana* Hsp18.2 heat shock gene (Takahashi and Komeda, 1989). The additional BamH1 site is located in the Hsp18.2 untranslated leader at nucleotide -710 of the Hsp18.2 translational start.

By restriction digestion with BamHI the 5' untranslated leader and 77 nuclectides of Hsp18.2 coding sequence were removed. The remaining

construct was called leaderless pTT19. A HindIII/BamHI fragment of this leaderless pTT19, containing only Hsp18.2 promoter sequence, was fused to a BamHI/EcoRI fragment containing the entire ATH1 cDNA sequence, which results in a transcriptional fusion of Hsp18.2 promoter with ATH1 5' untranslated leader and coding sequence. The BamHI and EcoRI sites were created by PCR mutagenesis, resulting in a BamHI restriction site at the beginning of the ATH1 cDNA sequence and a EcoRI restriction site immediately downstream of the TAA stop codon. The resulting HindIII/EcoRI fragment was inserted into the unique HindIII/EcoRI restriction sites of pWP90 vector. (see Figure 2) and this new construct was then partially digested with HindIII and EcoRV restriction enzymes. The largest HindIII/EcoRV restriction fragment was then inserted into HindIII/SmaI cut binary vector pBIN 19 (Frisch *et al.*, 1995). This construct was called HspH1.

A transcriptional fusion between Hsp18.2 promoter and ATH1 coding sequence without leader sequence was also made. In ATH1 cDNA an extra BamHI site was created by PCR mutagenesis immediately upstream of the translational start. Digestion of this BamHI site combined with digestion of the unique XhoI site in ATH1 cDNA results in an fragment of approximately 680 nt, containing ATH1 coding sequence. This fragment of 680 nucleotides was swapped with an approximately 980 nucleotides large fragment that is formed after digestion of HspH1 with BamHI/XhoI restriction enzymes. This results in HspH1B, a transcriptional fusion between leaderless ATH1 coding sequence and the

Hsp18.2 promoter.

Both HspH1 and HspH1B were transformed to competent *Agrobacterium tumefaciens* LBA4404 cells. *Arabidopsis* lines (C24 ecotype) were transformed as described below.

2.4 Fusion of the pea plastocyanin promoter to the ATH1 open reading frame

A transcriptional fusion between pea plastocyanin promoter and ATH1 coding sequence can be made by insertion of ATH1 coding sequence into the unique BamHI and SalI resriction sites of pVDH275 (Pwee and Gray, 1993; Last and Gray, 1989) (see also Figure 4). In ATH1 coding sequence additional SalI (immediately upstream of ATH1 start ATG) and BamHI (immediately after ATH1 stop TAA) restriction sites can be created by PCR mutagenesis. The resulting construct in which ATH1 coding sequence is inserted between pea plastocyanin promoter and *Agrobacterium* nos terminator, can be transformed to *Agrobacterium tumefaciens* cells, followed by plant transformation.

Introduction of extra ATH1 copies in *Arabidopsis*

Extra copies of ATH1 can be introduced in *Arabidopsis* plants by transforming them with extra ATH1 loci containing ATH1 promoter and ATH1 coding sequence. This can be done by fusion of the approximately 1000 nuclectides large SnaBI/NcoI fragment of ATH1 cDNA to the approximately 250 nucleotides large SstI/EccRI restriction fragment of pBI101.1, containing the

Agrobacterium nos terminator (Jefferson *et al.*, 1987). The resulting fragment can be fused to the approximately 3.8 Kb large NcoI restriction fragment of ATH1 genomic clone (Quaedvlieg *et al.*, 1998). The so formed approximately 4750 nucleotides large NcoI/EcoRI fragment, containing ATH1 promoter, ATH1 coding sequence and nos terminator, can be inserted into NcoI/EcoRI cut pMTL23 cloning vector (Chambers *et al.*, 1988). A StuI/EcoRI restriction fragment of the resulting construct can then be inserted into EcoRI/SmaI cut pMOG23 binary vector, *Agrobacterium* cells can be transformed, subsequently followed by plant transformation.

EXAMPLE 3

Influencing flowering characteristics using a CaMV 35S promoter/ATH1 gene fusion

Measurement of flowering time

Flowering time was measured by counting the number of leaves, excluding the cotyledons, in the rosette at the time the flower bud was visible. A close correlation between leaf number and flowering time has been previously demonstrated (Koorneef et al., 1991; Bagnall (1993)).

Overexpression of ATH1 leads to delayed flowering.

In order to gain more insight into the role of ATH1 in plant development, the full length ATH1 cDNA sequence was fused to the constitutive 35S promoter of cauliflower mosaic virus and the 35S::ATH1 chimeric gene so produced was transformed into *Arabidopsis* Col-0 ecotype via the

vacuum infiltration method. Six independent primary transformants were obtained.

All these transgenic lines were selfed. From each independent transgenic line 40 individual seeds were germinated on soil and scored for altered phenotypes compared to wild-type plants. Four out of six lines showed a phenotype altered in respect of flowering time. In three of these lines all plants were late flowering (about 14 rosette leaves up to flowering compared with about 10 rosette leaves in wild-type Col-0 plants). In the remaining line about 85 % of the plants showed this same late flowering phenotype, while 15 % of the plants showed an early flowering phenotype (after about 7 rosette leaves), as tested due to the absence of ATH1 RNA. These early flowering plants also show a terminal flower phenotype, often with incomplete flowers and mutant flower organs.

EXAMPLE 4

Early flowering by antisense expression of ATH1

Like ectopic overexpression of ATH1, inhibiting the ATH1 gene function can also can be used to influence time of flowering. Inhibition of gene function was effected by constitutive overexpression of antisense ATH1.

Full length antisense ATH1 cDNA was fused to the constitutive 35S promoter of cauliflower mosaic virus and the 35S::antisense ATH1 chimeric gene so produced was transformed into *Arabidopsis* C24 ecotype via the Valvekens root transformation protocol. Twenty-two independent transformants were obtained and all of them were selfed. From each line 10 individual seeds were germinated on soil and scored for altered phenotypes compared to wild-type C24

plants. In five of these lines, the plants showed an early flowering phenotype: flowering started after formation of between six and ten rosette leaves compared to about twenty leaves in wild-type plants.

EXAMPLE 5

Altering flowering time in *Nicotiana tabacum* by overexpression of ATH1

As in *Arabidopsis*, ectopic overexpression of ATH1 cDNA (driven by the 35S promoter of cauliflower mosaic virus) in tobacco (*Nicotiana tabacum* cv. Samsun) also led to a delay in flowering time compared to wild-type tobacco. In 35S::ATH1 tobacco plants, flowering was delayed by weeks or months. These plants were also dwarfed. This dwarf habit, like the flowering phenotype, is clearly correlated with the level of expression of the transgene. In the severest case plants did not flower at all and only reached one-fifth of their normal height, whereas in less severe cases plants were delayed in flowering for only one or two weeks and reached about four-fifth of their normal height. Leaf number and shape were normal in all these transformed plants.

EXAMPLES 6-8

The following Examples illustrate the effect of GA on transgenic plants according to the invention. As noted above, ATH1 overexpression effectively represses bolting (floral induction). We hypothesised that ATH1 may be a repressor of GA synthesis or the GA response pathway (we think the former). The following Examples demonstrate and support this hypothesis.

General Methods

Tobacco plants (*Nicotiana tabacum* L. cv. Samsun NN) were transformed using the leaf disk procedure (Horsch et al., 1985). Transgenic plants were selected on MS-medium (Murashige and Skoog, 1962) containing 300 mg/ml kanamycin and 2% sucrose. After transfer to soil plants were grown in a greenhouse at 22 °C under a light regime of 16 hours daylight when necessary supplemented with artificial light. The effects of gibberellin (GA) were tested by foliar applications (spraying) of 100mM GA3 in a solution containing 100ml/l of Triton X-100. Control plants were sprayed with a solution containing only 100ml/l of Triton X-100. Spraying began 60 days after sowing when the wild-type plants were approximately 5 cm tall and the 35S CaMV::ATH1 plants approximately 2.5 cm tall, and continued at 3- to 4- day intervals. Plant height was measured every 3 to 4 days and this will be continued until the onset of flowering, as determined by the appearance of flower primordia.

EXAMPLE 6

Constitutive overexpression of sense ATH1 leads to delayed flowering.

6.1 ATH1 over-expression in tobacco

In order to express the ATH1 gene constitutively in transgenic plants, its coding region was put under the control of the 35S CaMV promoter and the resulting construct was transformed to tobacco (*Nicotiana tabacum* cv. Samsun NN). Forty independent kanamycin-resistant plants were obtained, of which only five showed detectable transgene expression. ATH1 mRNA levels varied from high in H10E#4, #10 and #30 plants to intermediate/low levels in

H1OE#35 and #37 plants. Depending on ATH1expression level, flowering of these plants was delayed by weeks up to months, when compared to wild-type plants, which flower after 3-5 months depending on the season. In the severest case (H1OE#4) plants never flowered until senescence (>15 months after sowing). H1OE#10 and #30 plants, which show high ATH1 expression, flowered after 15 months, while plants showing the intermediate/low overexpression, H1OE#35 and 37, did not flower until after 6 months. As well as altered flowering-time phenotype, ATH1 overexpressor plants show reduced stem growth, resulting in dwarfed plants. Here there is a clear correlation between severity of the dwarf growth phenotype and the level of transgene expression (see Figure 5). In the severest case plants only reach about one-fifth of their normal height. The leaf number varies from two times higher than wild type to normal in all transgenes.

6.2 ATH1 overexpression can be reversed by GA3

ATH1 overexpression phenotypes can be reversed to a wild-type phenotype by application of GA3. Foliar application of GA3 to the tobacco plants of Example 6.1 (spraying of 100 mM GA3 at three to four day intervals) results in complete restoration of the wild-type stem length (Figure 6). This holds also true for the late-flowering phenotype (data not shown).

EXAMPLE 7

ATH1 over-expression in *Arabidopsis*

In order to gain more insight into the role of ATH1 in plant development, the full length ATH1 cDNA was fused to the constitutive 35S promoter of cauliflower mosaic virus

and the 35S::ATH1 chimeric gene was transformed into *Arabidopsis* via the vacuum infiltration method. Six independent primary transformants could be obtained and all transgenic lines were selfed. From each independent transgenic line 40 individual seeds were germinated and scored for altered phenotypes compared to wild type plants. Four out of six lines showed an altered phenotype in respect of flowering time. Seeds from these lines did not germinate well and if they did plants were arrested in a seedling stage. Both effects could be overcome by transferring the plants to growth medium containing 10-5 M GA3 and growing them on this medium for three days. Once rescued and transferred to soil plants developed normally, except for a late flowering phenotype. Under short day conditions transgenic plants form much more rosette leaves (vegetative leaves) than wild type plants (about 40 rosette leaves and 100 days after germination, and plants are still not flowering, compared to about 30 rosette leaves in wild-type plants until flowering). Under LD conditions in most of these plants a partial generative to vegetative reversion occurs, shown by the formation of aerial rosettes (vegetative leaves) on the inflorescence stem. Plants (C24 ecotype) containing an extra copy of the ATH1 cDNA under control of the Hsp18.2 heat shock promoter (HspH1B plants) also show a late-flowering phenotype. Even without a heat shock (it is known that this promoter has a basal activity without induction) plants harboring this construct flower much later under LD conditions than wild-type plants (30.5 rosette leaves formed in wild-type vs. 61 rosette leaves formed in HspH1B plants - see Figure 7).

EXAMPLE 8

Early flowering by antisense expression of ATH1

Like ectopic overexpression of ATH1, knocking out the ATH1 gene function can also give insights into the function of ATH1 in plant development. Knocking out gene function was established by constitutive overexpression of antisense ATH1. Full length antisense ATH1 cDNA was fused to the constitutive 35S promoter of cauliflower mosaic virus and the 35S::antisense ATH1 chimeric gene was transformed into *Arabidopsis* C24 ecotype via the Valvekens root transformation protocol. Twenty-two independent transformants were obtained and all of them were selfed. From each line 10 individual seeds were germinated on soil and scored for altered phenotypes compared to wild type C24 plants. In five of these lines plants showed an early-flowering phenotype: flowering started after formation of about ten rosette leaves compared to about thirty leaves in wild type plants (see Figure 7).

Example 9

Shade avoidance response

When plants grow in close proximity shade avoidance syndrome, in which plants react to lowered red/far-red ratios of light caused by filtering out red light by leaf canopy, is manifested. This results in a rapid and dramatic increase in the extension growth of stems and petioles at the expense of leaf growth, storage organ production, and reproductive development, thereby causing a decrease in harvest index (where harvest index is expressed as leaf biomass as a proportion of total biomass).

The shade avoidance response is thought to be predominantly mediated by phytochrome B and overexpression of phytochrome has been shown to eliminate the shade avoidance response, resulting in an increase of harvest index of field-grown tobacco (Robson *et al.*, 1996).

Lack of phytochrome B also leads to loss of shade avoidance response and under inductive conditions this even results

in a reduction of stem elongation compared with non-inductive conditions.

In the laboratory, situations causing the shade avoidance response can mimicked by addition of different fluence rates of far-red light (Frc) to continuous white light (Wc). Under these conditions wild-type *Arabidopsis* plants (C24 wt) show a typical shade avoidance response (elongated hypocotyls in Wc + Frc compared with hypocotyl length in Wc only), whereas the phytochrome B photoreceptor mutants, which lack the shade avoidance response, exhibited an opposite response (decrease of hypocotyl length). The antisense AtH1 plants also show a reduction in hypocotyl length and in the most severe antisense plants (asAtH1#3) this reduction is similar to that seed in phyB mutants. So, it can be concluded that like lack of active phytochrome B loss of Ath1 results in loss of the shade avoidance response.

Shade avoidance analysis of antisense AtH1 plants (asAtH1), C24 wild-type plants (C24 wt) and the phy B photoreceptor mutant (phyB) is listed below. Seedlings where grown for 2 days in continuous white light followed by 4 days in the same light regime or by 4 days in white light supplemented by far-red light. The hypcototyl length was measured after 6 days of growth. The following results were generated.

C24wt	Wc	=	hypocotyl length	5.5 mm
	Wc + Frc	=	hypocotyl length	7.5 mm
Phy B	Wc	=	hypocotyl length	9.5 mm
	Wc + Frc	=	hypocotyl length	7.3 mm
Anti-sense AtH1#3	Wc	=	hypocotyl length	9.0 mm
	Wc + Frc	=	hypocotyl length	5.0 mm
Anti-sense	Wc	=	hypocotyl length	9.8 mm

AtH1#7	Wc + Frc	=	hypocotyl length	8.0 mm
Anti-sense	Wc	=	hypocotyl length	7.5 mm
AtH1#23	Wc + Frc	=	hypocotyl length	7.0 mm

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Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco

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Wilson, R.N., Heckman, J.W., and Sommerville, C.R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant. Phys. 100, 403-408.

WE CLAIM:

1. A plant gene construct comprising a complete or partial DNA sequence coding for an ATH1 gene product under the control of a promoter functional in plants.

2. A plant gene construct as claimed in claim 1 in which the promoter is heterologous.

3. A plant gene construct as claimed in claim 2 in which the promoter is constitutive.

4. A plant gene construct as claimed in claim 2 in which the promoter is inducible.

5. A plant gene construct as claimed in any of claims 1-4 in which the complete or partial DNA sequence is homologous with the DNA sequence shown in Figure 1.

6. A plant gene construct as claimed in any of claims 1-5 which is adapted to express RNA antisense to RNA produced by the ATH1 gene.

7. A plant gene construct as claimed in any of claims 1-5 which is adapted to express RNA homologous to RNA produced by the ATH1 gene.

8. A plant cell transformed with a DNA construct claimed in any of claims 1-7.

9. A plant cell as claimed in claim 8 adapted to express RNA that produces recombinant ATH1 protein in the cell.

10. A plant cell as claimed in claim 8 adapted to express RNA that inhibits the production of ATH1 protein in the cell.

11. A plant comprising transformed plant cells as claimed in any of claims 8-10.

12. A plant as claimed in claim 11 which is a crop plant.

13. A plant as claimed in claim 12 which is rice, maize, wheat, barley, oats, rye, lettuce, endive, oilseed rape (canola), sugar beet, sunflower, soya or sorghum.

14. A plant as claimed in either of claims 12 or 13 adapted to produce recombinant ATH1 protein.

15. A process for modifying flowering in plants which comprises transforming the plants with a construct as claimed in any of claims 1-5.

16. A process as claimed in claim 15 whereby the flowering process in plants is promoted by transforming the plants with a construct claimed in either of claims 6 or 7 that inhibits the production of ATH1 protein.

17. A process as claimed in claim 15 whereby the flowering process in plants is retarded by transforming the plants with a construct claimed in claim 7 that promotes the production of recombinant ATH1 protein.

18. A process for inhibiting over-expression of ATH1 in plants claimed in claim 14 which comprises treating the plants with a gibberellin.

19. A process as claimed in claim 18 in which the gibberellin is A3 or A4/A7.

20. A plant DNA construct comprising the ATH1 promoter linked to heterologous DNA so as to cause transcription thereof in plant cells.

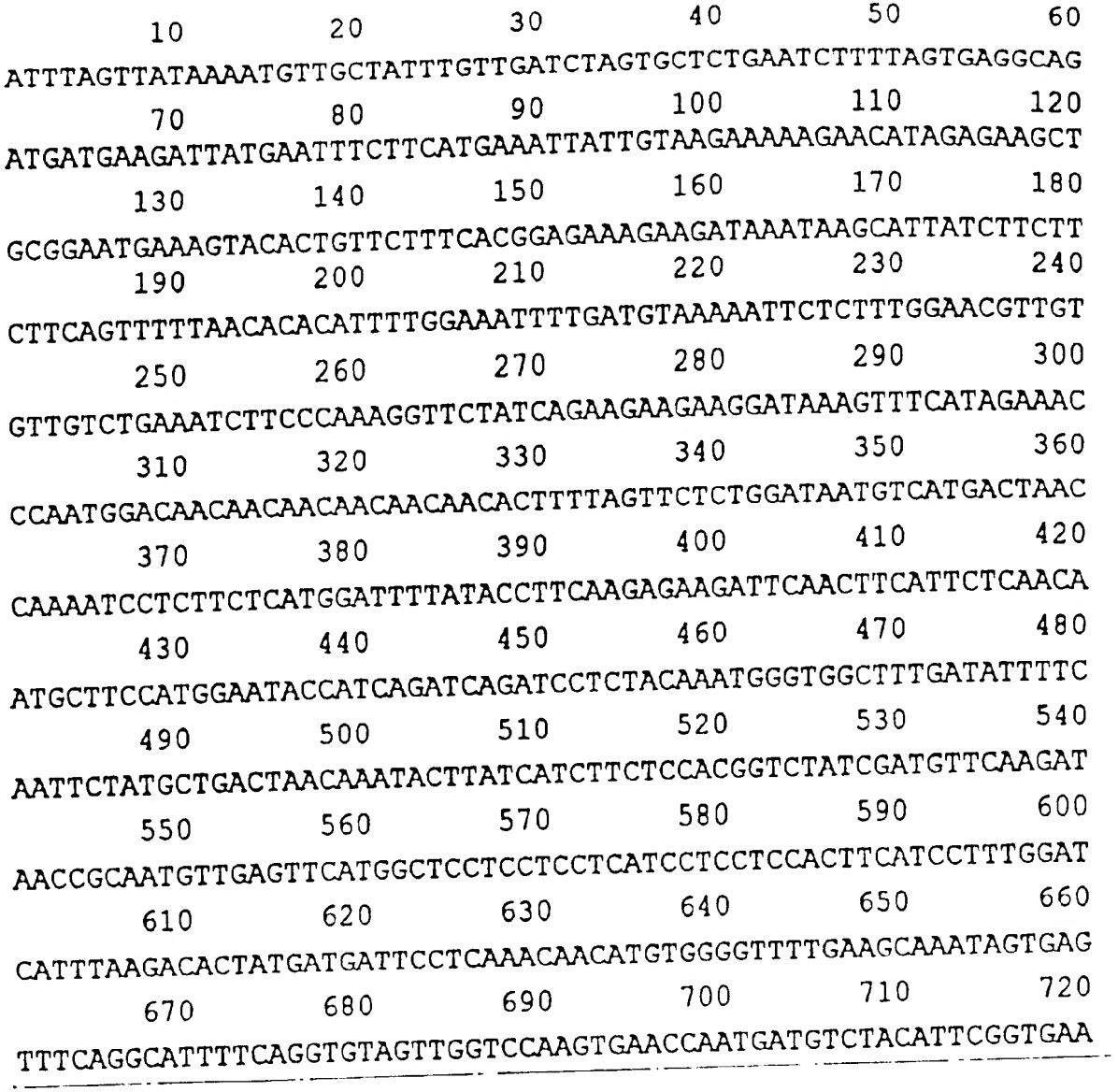
21. Plant cells transformed with a construct claimed in claim 20.

22. A plant lacking a shade avoidance response comprising: a plant transformed with a transgene wherein said transgene induces a shade avoidance response in said transformed plant.

23. A plant according to claim 22 wherein said transformed plant is formed from a wildtype plant which has a shade avoidance response.

24. A method of producing a transgenic plant that lacks the shade avoidance response of a wildtype plant, comprising:
forming a construct having a complete or partial DNA sequence coding for an ATH1 gene product;
transforming said wildtype plant material with said construct; and
forming plants therefrom.

FIG.1



PCT/IB98/00821

FIG. 1(CONTD)

```
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GAAGATTTCCCGTTTCTAATTTCGAATAAAAGAAACAATGAGCTTTCATTGAGTCTTGCA
        790         800         810         820         830         840
TCAGATGTTTCTGATGAATGCTCGGAGATAAGTCTTTGTGCAGCTACAAGATTAGCCTCA
        850         860         870         880         890         900
GAGCAAGCTTCTTGCAGCAGCAAAGACATTTCTAATAACGTTGTTACTCAAGGTTTCTCT
        910         920         930         940         950         960
CAACTTATATTTGGCTCAAAATACCTTCACTCTGTTCAAGAAATACTATCTCATTTCGCC
        970         980         990        1000        1010        1020
GCATACTCGCTCGATTATTCATCTCGAGGAACCGAGTCAGGAGCTGCTAGTTCAGCCTTT
       1030        1040        1050        1060        1070        1080
ACTTCACGTTTTGAGAATATAACTGAGTTTCTTGATGGTGATTCTAATAACTCGGAGGCG
       1090        1100        1110        1120        1130        1140
GGTTTCGGATCTACATTTCAAAGGAGAGCATTAGAAGCAAAGAAAACCCATCTCTTGGAT
       1150        1160        1170        1180        1190        1200
CTTCTTCAAATGGTGGATGATCGATATAGTCATTGCGTAGATGAGATTCATACGGTTATA
       1210        1220        1230        1240        1250        1260
TCAGCGTTCCATGCTGCAACCGAGTTAGATCCACAGTTACACACCCGGTTTGCCCTCCAA
       1270        1280        1290        1300        1310        1320
ACCGTTTCCTTCTTATACAAGAACCTGAGAGAGAGAATCTGCAATAATATAATCTCTATG
       1330        1340        1350        1360        1370        1380
GGATCTGTATTGGAGAGAGGCAAAGACAAGACTCAAGAAACCTCTATGTTCCACCAGCAT
       1390        1400        1410        1420        1430        1440
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TGCCTTCTTCAGCAGCTGAAACGAAAGAACCATCAGATTTGGAGACCTCAACGAGGTTTG

1450 1460 1470 1480 1490 1500

CCTGAGAAATCTGTTTCGGTTCTACGGAATTGGATGTTCCAAAACTTCCTTCACCCTTAC

1510 1520 1530 1540 1550 1560

CCGAAAGATTCGGAGAAACATCTTCTAGCTATACGAAGTGGCTTGACAAGAAGTCAGGTA

1570 1580 1590 1600 1610 1620

TCAAACTGGTTTATAAATGCGCGGGTTAGGCTATGGAAGCCGATGATAGAAGAGATGTAT

1630 1640 1650 1660 1670 1680

GCGGAAATGAACAAGAGGAAGCTCAATAACAGTCACATTCAACCCAACGGACCAACTCTT

1690 1700 1710 1720 1730 1740

CGAATGCCAAAATCTGTTATGATGAGCCAAGCAATGCATAAATAAGACAACAATTGTGTT

1750 1760 1770 1780 1790 1800

TACCAACTTTGTGATAATTAGGCAATTGCTACTCTATGATTGCCCAAAACCTAAACCATG

1810 1820 1830 1840 1850 1860

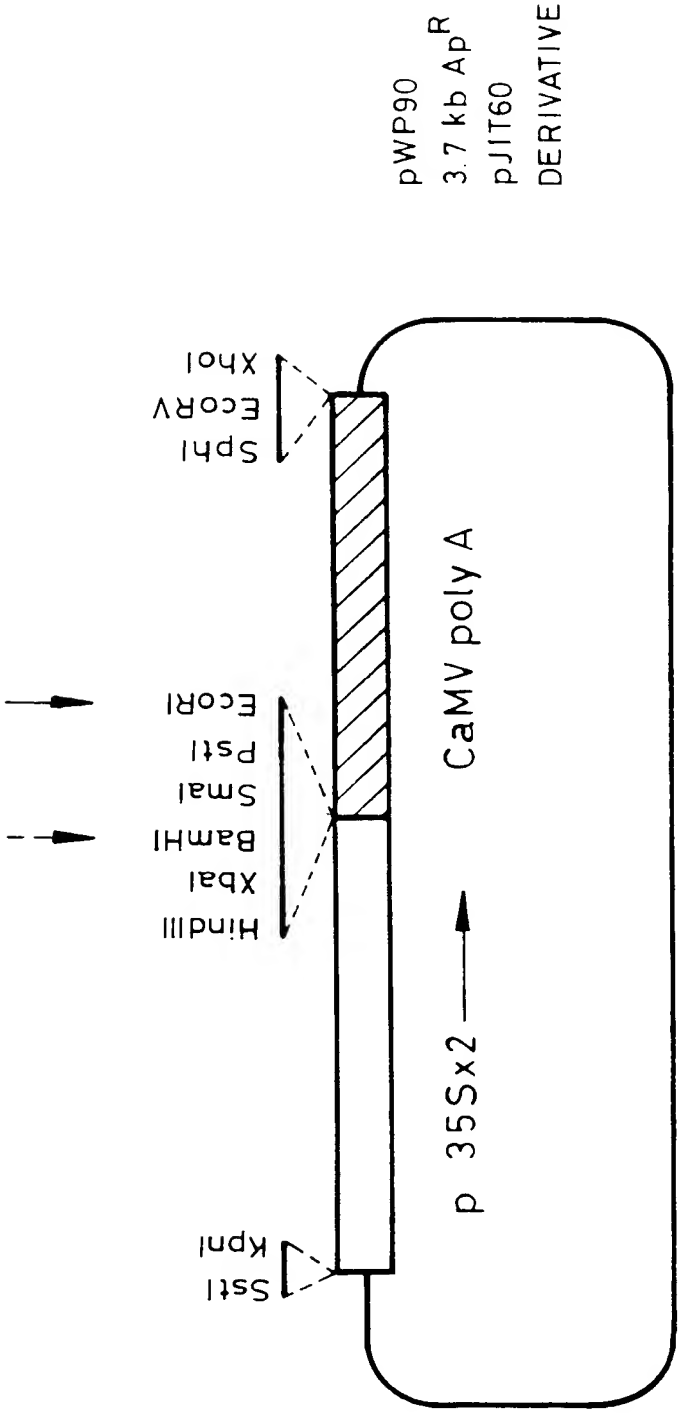
TACGACTATCATTACGTATGTTATAATTGTATATACAACTCCTTTATCTTTGACTATTTC

1870 1880 1890 1900

ATTTTATTAAAAAAAAAAAAAAAAAA

FIG. 1 (CONTD)

FIG. 2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XhoI
EcoRV
SphI
EcoRI
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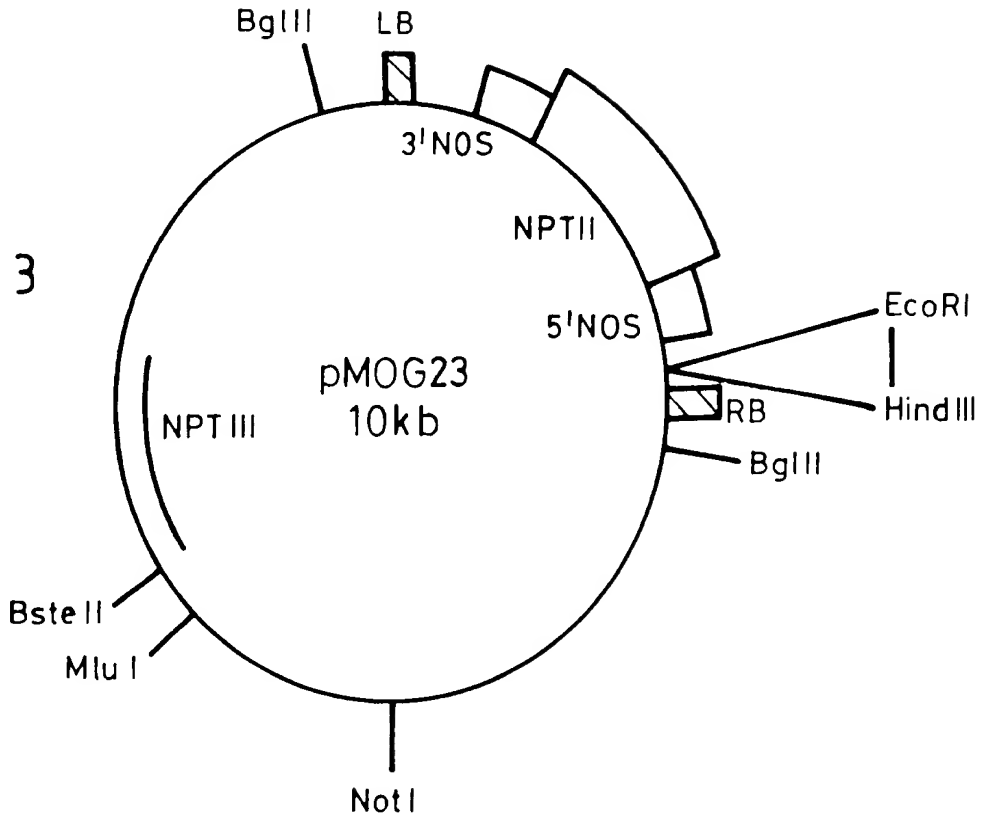
HindIII
SstI
KpnI
p 35Sx2
CaMV poly A

FIG. 3



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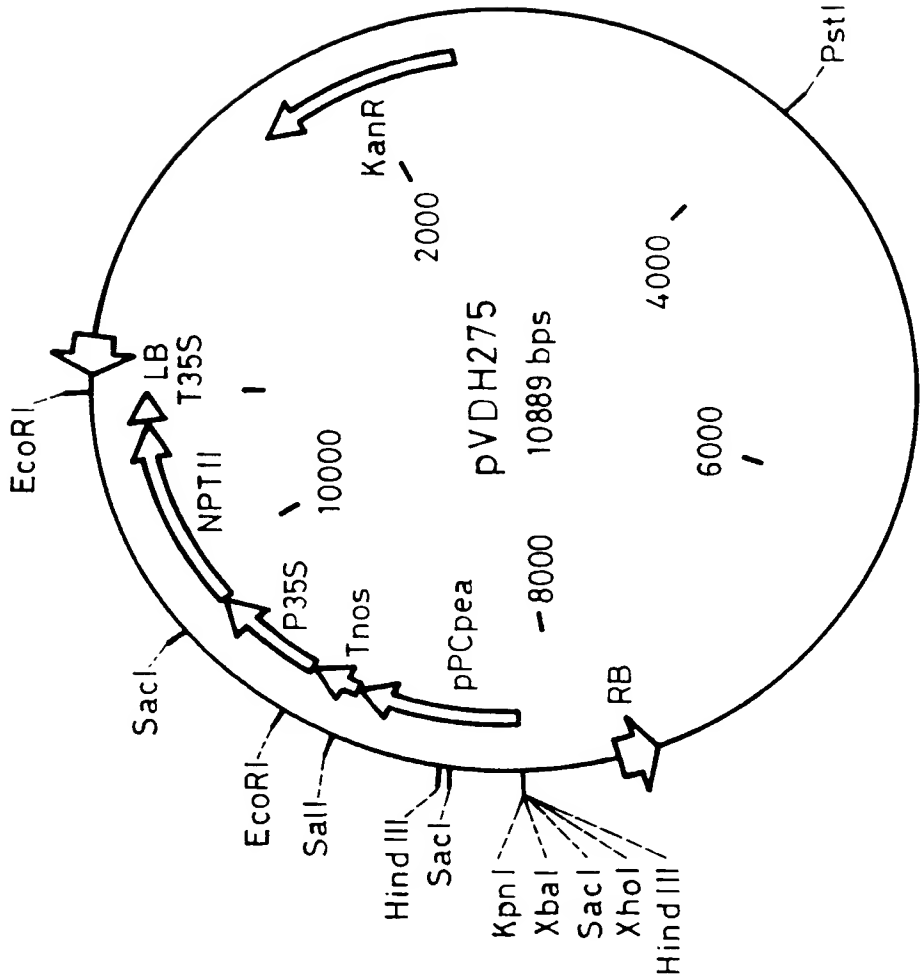
SacI

FIG. 4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pPCpea
RB
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EcoRI
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SalI
HindIII
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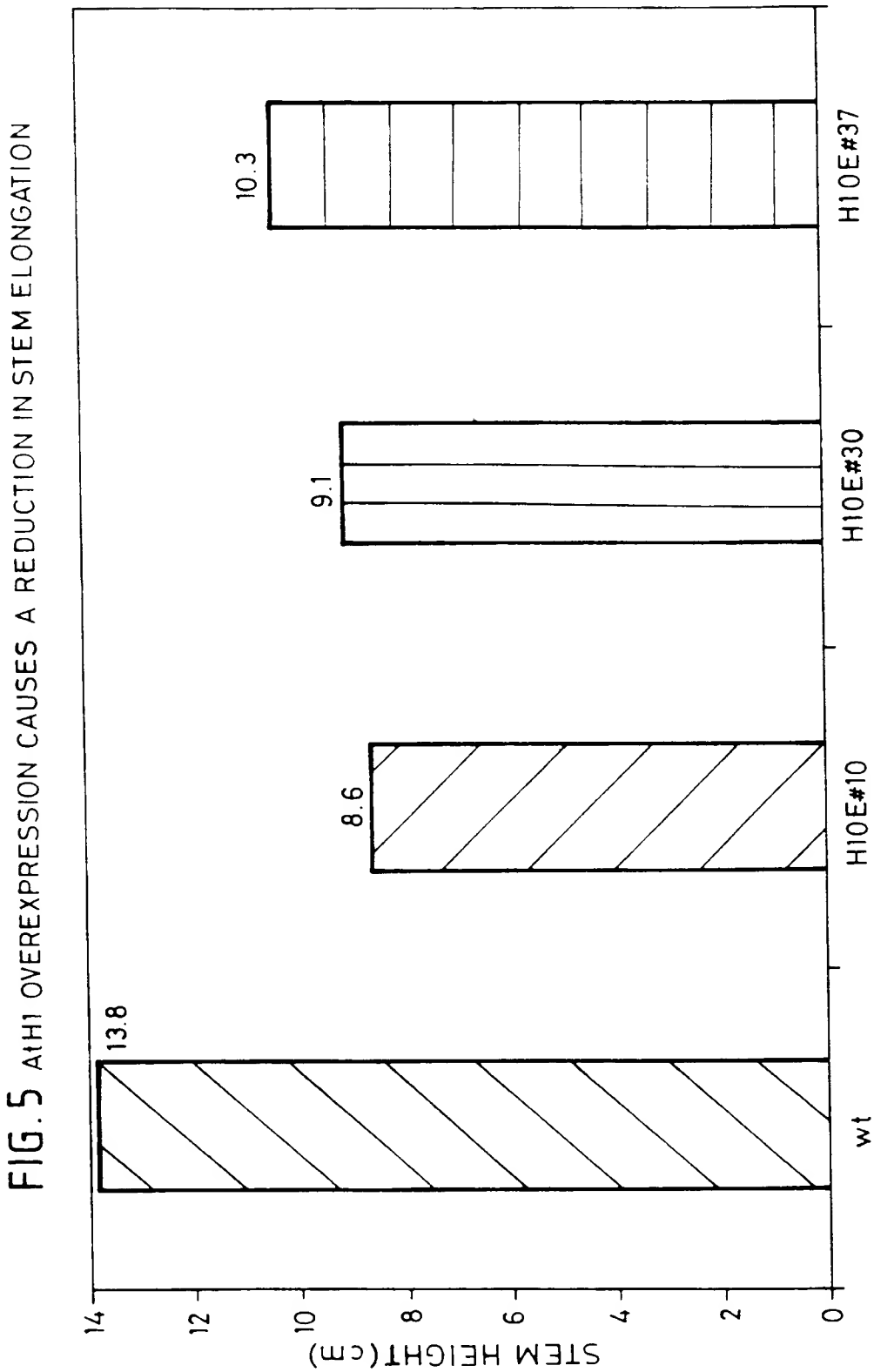


FIG. 5 AtH1 OVEREXPRESSION CAUSES A REDUCTION IN STEM ELONGATION

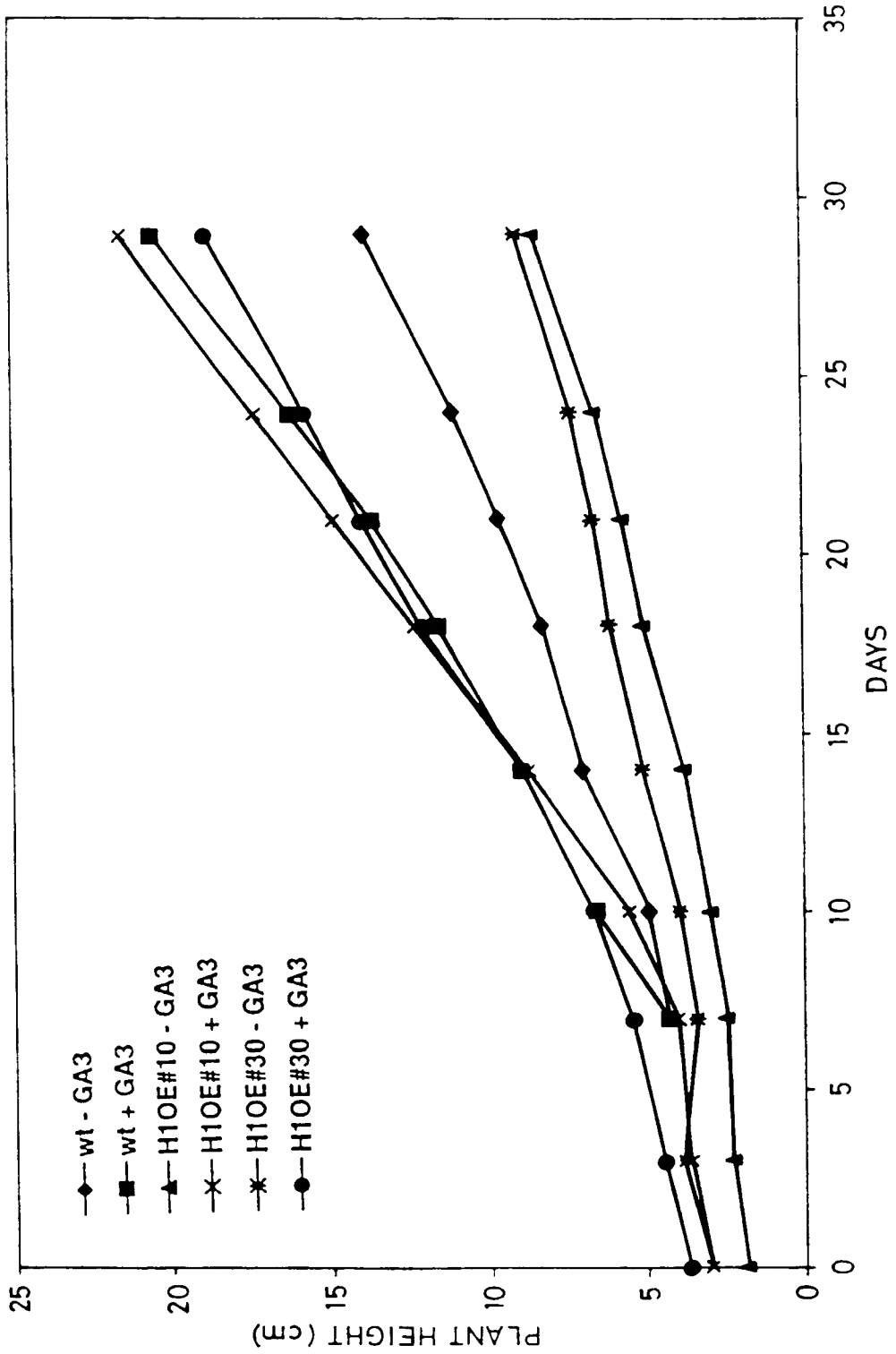
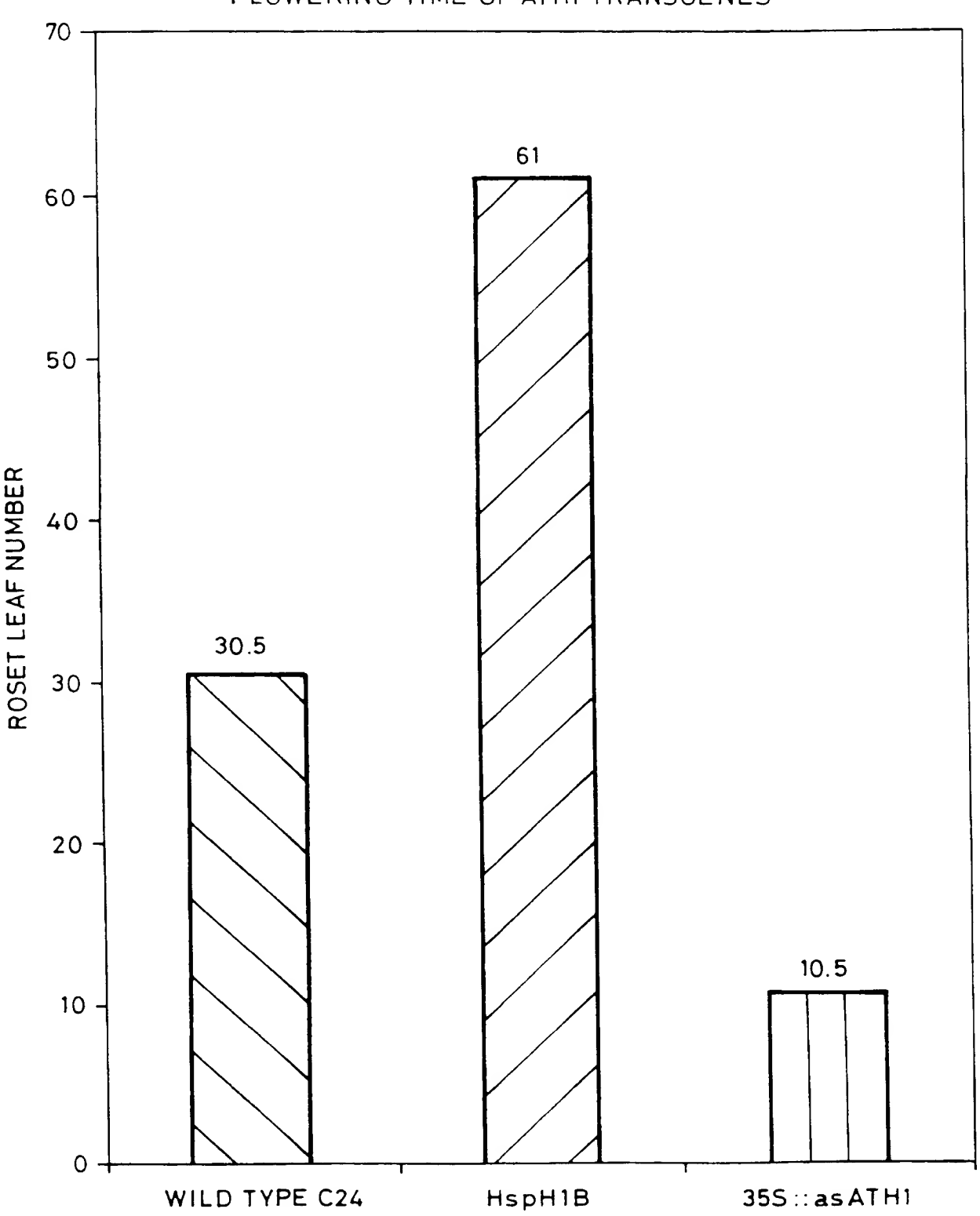


FIG.6 REVERSION OF AtH1 OVEREXPRESSION PHENOTYPE BY GA3

FIG. 7


FLOWERING TIME OF ATH1 TRANSGENES
ROSET LEAF NUMBER
70
60
50
40
30
20
10
0
30.5
61
10.5
WILD TYPE C24
HspH1B
35S::asATH1

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00821

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N15/11 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	PAUL R. ROBSON ET AL: "Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene." NATURE BIOTECHNOLOGY, (1996) 14/8 (995-998). ISSN: 0733-222X CODEN: NABIF, XP002075914 cited in the application	22,23
A	see the whole document	24

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1998	21/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kania, T

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00821

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	QUAEDVLIEG N ET AL: "The homeobox gene ATH1 of Arabidopsis is derepressed in the photomorphogenic mutants cop1 and det1." PLANT CELL, (1995 JAN) 7 (1) 117-29. JOURNAL CODE: BJU. ISSN: 1040-4651.. XP002075915 cited in the application * see the whole document, esp. p.124 l. col. 2.par. - r.col. 1.par *	1-14,20, 21
A	LINCOLN C. ET AL: "A knotted1-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants." PLANT CELL, (1994 DEC) 6 (12) 1859-76. JOURNAL CODE: BJU. ISSN: 1040-4651., XP002075916 see the whole document	1-14,20, 21
A	AOYAMA T. ET AL.: "Ectopic expression of the Arabidopsis transcriptional activator Athb-1 alters leaf cell fat in tobacco" THE PLANT CELL, vol. 7, no. 11, November 1995, pages 1773-1785, XP002023729 see the whole document	1-14,20, 21
A	CHUCK G ET AL: "KNAT1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis." PLANT CELL, (1996 AUG) 8 (8) 1277-89. JOURNAL CODE: BJU. ISSN: 1040-4651., XP002075917 see the whole document	1-14,20, 21
A	WO 96 14414 A (JOHN INNES CENTRE ;COUPLAND GEORGE MICHAEL (GB); PUTTERILL JOANNA) 17 May 1996 cited in the application see the whole document	15-19
A	WO 97 10339 A (JOHN INNES CENTRE ;BRADLEY DESMOND JOSEPH (GB); CARPENTER ROSEMARY) 20 March 1997 * see the whole document, esp. p.17 ff. *	15-19

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INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/IB 98/00821

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROVENIERS M (REPRINT) ET AL: "The Arabidopsis homeobox gene ATH1 and floral transition" DEVELOPMENTAL BIOLOGY, (15 JUN 1997) VOL. 186, NO. 2, PP. A49-A49. PUBLISHER: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0012-1606., XP002075918 see abstract	1-24

1

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INTERNATIONAL SEARCH REPORT

formation on patent family members

Interr nal Application No
PCT/IB 98/00821

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9614414 A	17-05-1996	AU 3809795 A	31-05-1996
		CN 1171817 A	28-01-1998
		EP 0789765 A	20-08-1997
WO 9710339 A	20-03-1997	AU 6939596 A	01-04-1997
		EP 0852622 A	15-07-1998